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Role of Polyclonal Cell Activation in the Initiation of Immune Complex-Mediated Pulmonary Injury Following Antigen Inhalation

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The lung, by virtue of its anatomic situation, provides environmental antigens with unique access to host lymphoid tissues. In order to better understand the biologic consequences of antigen inhalation, we developed an animal model in which soluble proteins are administered in aerosol form to rabbits. By labeling these proteins with fluorochrome dyes or radioactive isotopes, the uptake, distribution, and fate of such proteins can be demonstrated both morphologically and quantitatively. Prompt host-antibody responses can be demonstrated to inhaled antigen, but not to comparable amounts of ingested antigen.

Repeated administrations of antigen aerosol to immune animals produced little injury; in contrast, administration of aerosols containing phytohemagglutinin or concanavalin A (Con A), plant lectins which activate leucocytes in a polyclonal fashion, induced a diffuse interstitial pneumonitis. When immune animals inhaled antigen plus Con A, devastating pulmonary necrosis was induced, in association with localized deposits of immune complexes containing antigen, antibody and complement. Such necrotic injury healed by scarring within 4 weeks. The necrotizing injury could be prevented by either decomplementation with cobra venom factor, or through inhibition of leucocyte responsiveness to Con A by administration of cholera toxin, a cAMP agonist.

These studies indicate that antigen inhalation may serve as an important means of establishing "natural" immunity to environmental agents, but also may lead to severe pulmonary injury and fibrosis where the agents inhaled act not only as antigens but as polyclonal leucocyte activators as well.

Introduction

Certain forms of environmental lung disease provoked by inhaled substances are thought to result from immunologically mediated inflammatory injury to the pulmonary parenchyma; such diseases have been termed "allergic alveolitis" or, alternatively, "hypersensitivity pneumonitis." In order to better understand the pathogenesis of such injury, animal models have been studied in which protein antigens, polyclonal cell activators derived from plants, or other organic materials have been administered in aerosol form to normal or immune animals.

Inhalation of bovine serum albumin (BSA), a protein antigen, causes little, if any, injury in BSA-immunized rabbits, presumably because it does not have access to humoral antibody (*1*). However, we have observed that inhalation challenge with mixtures of antigen plus concanavalin A (Con A), a polyclonal cell activator, produced a severe necrotizing pneumonitis in immune recipients, in association with localized deposits of immune complexes of inhaled antigen, host antibody and complement (*2, 3*). We now present evidence that this enhancement of immune complex formation in the lung by

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inhaled Con A occurs as a direct consequence of Con A's ability to stimulate lymphocytes *in vivo*. To do this, we have employed purified cholera toxin (CT), a cAMP agonist which inhibits leucocyte stimulation by Con A *in vitro*.

First, we have compared three different regimens of *in vivo* CT administration for inhibition of Con A-induced stimulation of peripheral lymphocytes *in vitro*, and also for inhibition of Con A-induced inflammation in the skin. We then demonstrate that CT administration selectively inhibits cell-mediated, delayed hypersensitivity skin reactions but not the antibody-mediated vasculitis associated with the Arthus reaction. Finally, we show that when we block cell-mediated reactivity to Con A *in vivo*, we also block the ability of Con A to trigger immune complex reactions in the lung between inhaled antigen and humoral antibody. Lastly, these inhibitory effects of CT can be correlated with elevations in lymphocyte cAMP concentrations associated with CT administration.

Materials and Methods

New Zealand White male rabbits used in this study weighed 2-3 kg and were purchased from HARE (Hare, N.J.). The rabbits were housed in laminar flow rooms supplied with filtered air and given both rabbit chow and water ad libitum. All animals were immunized with BSA (Sigma, St. Louis, Mo.) incorporated into an alum precipitate as previously described (*1*). Each rabbit received a total of 50 mg BSA, 10 mg of protein initially, followed by 20 mg each on days 7 and 21, injected bilaterally in four equal parts at dorsal and gluteal sites.

Aerosols containing BSA and Con A were administered as previously described (*1*). Briefly, aerosols were generated in a DeVilbiss model 35A ultrasonic nebulizer containing a solution of 100 mg BSA and 100 mg Con A in 20 ml of PBS. Each aerosol was vented into a Plexiglas chamber into which two rabbits' heads could be inserted simultaneously. Each aerosol administration required approximately 60-90 min to complete, and was administered to each animal on three consecutive days. All animals were then sacrificed 24 hr after the last aerosol by an intravenous overdose of nembutal.

Conconavalin A

Con A was prepared from Jack bean meal (Difco, Detroit, Mich.) according to the method of Agrawal and Goldstein (*4*), modified as previously described (*3*). The purified Con A gave a single precipitin band by immunodiffusion in gel against rabbit anti-Jack bean meal antibody, and a single symmetrical peak by velocity sedimentation in a 10-40% sucrose density gradient.

Cholera Toxin

CT was purchased from Schwarz/Mann (Orangeburg, N.Y.). It was characterized by the supplier as giving a single band in disc electrophoresis. A 1-mg portion of lyophilized CT was initially resuspended in 1 ml H_2O, then diluted in saline to a final concentration of 100 or 50 μg/ml. Prior to injection, the diluted CT was filtered through a 0.22 Millipore filter (Bedford, Mass.). In search for optimal conditions of inhibition, several different regimes of CT administrations were employed in initial studies. In some experiments (protocol A), rabbits were injected via the marginal ear vein with 40 μg of CT/kg, 24 hr prior to the first aerosol, followed 48 hr later with a second IV injection of 20 μg/kg. In others (protocol B), daily injection of equal amounts were given in four consecutive days, beginning 24 hr prior to the first aerosol; the total daily dosage in the latter protocol was 80 μg/kg body weight or approximately 160 μg/animal. Finally, a third group of rabbits (protocol C) was given 40 μg of CT/kg IP, followed 48 hr later with a second injection of 20 μg/kg.

The amounts of CT employed in these studies were derived empirically in preliminary experiments. It is apparent from such studies that the amounts of CT required for suppression of immune effector reactions *in vivo* are near lethal values. Although no rabbit died in the experiments presented here (nor in most subsequent experiments carried out to date), we have on occasion seen animals receiving similar amounts of CT die within 24-48 hr of the first injection. At autopsy, the only obvious pathological findings in these animals was the occasional presence of fluid in the pleural cavities.

Lymphocyte Cultures

Rabbits were bled from the central artery of the ear and the peripheral lymphocytes isolated by the method of Boyum (*5*). The cells were washed twice with Hanks balanced salt solution (HBSS) (Microbiological Assoc., Rockville, Md.) and, if necessary, any remaining red blood cells lysed with 0.84% ammonium chloride. The lymphocytes were diluted in culture medium to give a suspension of 2×10^6 cells/ml. The culture medium employed consisted of RPMI 1640 (Microbiological Assoc.) containing 10*mM* HEPES buffer (Sigma, St. Louis, Mo.), 10% heat inactivated pooled normal rabbit serum, 2*mM* glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (Microbiological Assoc.).

Equal volumes (0.1 ml each) of diluted cells (2×10^5 cells) and of medium containing Con A were

added to each well of microculture plates (Coastar Plastics, Cambridge, Mass.). After incubation for 52-54 hr at 37°C in humidified air containing 5% CO_2, 0.05 ml of media containing 0.5 μCi of ^{3}H-thymidine (specific activity = 5.0 Ci/mmole, Amersham/Searle, Arlington Heights, Ill.) was added to each well. After 72 hr of total incubation time, the cells were harvested on Reeve Angel glass fiber filters (Whatman Inc., Clifton, N.J.) with an automatic cell harvester (Model 24V, Brandel, Rockville, Md.). The filters were air-dried and then placed in plastic scintillation vials (MiniVials, RPI, Rochester, N.Y.). A 5-ml portion of scintillation fluid (4g Omniscient to 1 liter toluene, RPI) were added to each vial and the samples counted in a Beckman LS 250 or LS 7000 liquid scintillation spectrophotometer. The degree of lymphocyte stimulation is expressed as the net cpm (total cpm minus cpm of unstimulated control cultures). Data are reported as the mean of triplicate cultures ± standard error of the mean.

Cyclic Nucleotide Determinations

Lymphocytes were isolated from arterial blood as described above and 0.5 ml aliquots containing 2×10^6 cells in HBSS were placed in 16 × 125 mm Pyrex test tubes, frozen rapidly in alcohol and Dry Ice, and then stored at −60°C. For assay, the cells were thawed slowly, 3 ml of ice-cold acetone added, and the cell suspension then allowed to stand for 15 min in an ice bath. The cell suspensions were then heated in a 60-70°C water bath for 3 min followed by centrifugation at 900 *g* for 15 min at 2-5°C. The supernatants were transferred to pyrex culture tubes and lyophilized.

Radioimmunoassay (RIA) for cAMP and cGMP was carried out under identical conditions, as previously described (*6*), by use of commercial reagents (Schwarz/Mann, Orangeburg, N.Y.). Briefly, lyophilized samples were then resuspended in 1.0 ml of cold (2-5°C) 0.05*M* sodium acetate buffer (pH 6.2) and 0.3 ml transferred to each of two 12 × 75 mm polypropylene culture tubes (one for cAMP and one for cGMP), followed by the addition of 0.1 ml of radioactive cyclic nucleotide [2-0-succinyl cyclic AMP (orcGMP) tyrosine methyl ester (125_I)] and 0.1 ml of rabbit IgG anti-cAMP or anti-cGMP to each tube. After mixing, all RIA tubes were held at 4°C for 16-20 hr followed by addition of 2.5 ml of 60% saturated ammonium sulfate to each tube. After 30 min, the tubes were centrifuged at 900*g* for 30 min at 2-5°C. The supernatants were decanted and any excess fluid removed with a cotton-tipped applicator. The precipitate radioactivity was measured in a Beckman 310 gamma spectrophotometer; the efficiency of ^{125}I activity measurements was determined to be 75%. For each assay, standard curves were constructed using known amounts of cyclic nucleotides (ranging from 0.05 to 10.0 pmole/tube). These curves were then used to relate the amount of cyclic nucleotide in unknown samples as a function of precipitated radioactivity.

Skin Tests

Rabbits were skin-tested 20-24 hr prior to sacrifice. All skin tests were done on the animal's left side, which had been previously shaved. Arthus skin tests were induced by intradermal injection of 0.05 ml of BSA dissolved in PBS at a final concentration of 1 mg protein/ml. Cell-mediated inflammatory skin reactions were induced by intradermal injection of 0.05 ml of Con A (200 μg Con A/ml PBS). In some instances, where negative reactions were anticipated, sterile india ink (1%) was added to the Con A and BSA skin test solutions. The india ink provided a marker for the positive identification of noninflammatory skin test sites; by itself, the ink caused no inflammatory response. After sacrifice, each test site was excised and fixed in formalin for subsequent processing for histologic examinations.

Histology

All tissue was fixed in formalin and paraffin-embedded tissue sections stained with hematoxylin and eosin. Lungs were inflated with isotonic buffered dormalin at a hydrostatic pressure of 10 cm H_2O. Sections were taken from all lobes; each section extended from the hilar region out to the periphery. Injury was judged and graded on the extent or degree of inflammatory change, and also in terms of the presence or absence of parenchymal necrosis.

Results

Effects of CT on Peripheral Blood Lymphocytes (PBL)

The effects of CT *in vivo* were examined by using three alternative administrative protocols. In protocol A, a total of 60 μg CT/kg of body weight was injected intravenously, two-thirds being given at time 0 and the remaining one-third at 48 hr; in protocol B, a total of 80 μg CT/kg of body weight was injected intravenously, given daily starting at time 0 as four equally divided doses; in protocol C, a total of 60 μg CT/kg body weight was injected intraperitoneally, two-thirds at time 0 and the remaining one-third at 48 hr. Peripheral blood lymphocytes were isolated from bleedings obtained at daily intervals, and tested

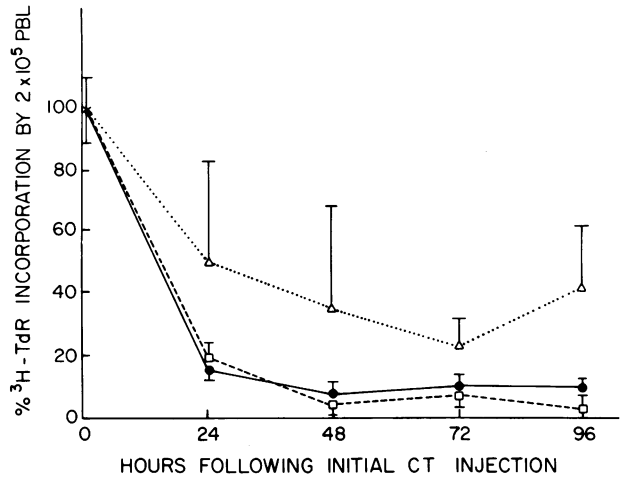


FIGURE 1. Effects of CT administration *in vivo* on responsiveness of peripheral blood lymphocytes (PBL) to stimulation *in vitro* by optimal concentrations of Con A by three different protocols for CT administration: (●) protocol A, 60 μg CT/kg, IV; (□) protocol B, 80 μg CT/kg, IV; (Δ) protocol C, 60 μg CT/kg, IP. Results are plotted as percent of ^{3}HtdR incorporation observed prior to CT administrations (time = 0), as observed in PBL obtained 24, 48, 72, and 96 hr following the initial CT injection. Each point represents the mean vlaue observed, using triplicate assays, in each of three animals; vertical bars express the standard error.

for their ability to incorporate ^{3}H-TdR when stimulated with Con A *in vitro*.

All animals given CT intravenously showed a drop in PBL responsiveness within 24 hr following the initial injection (Fig. 1). Animals treated with CT via protocols A or B (intravenously) showed over a 90% inhibition in proliferative responses to Con A, and remained unresponsive throughout the remainder of the experiment. On the other hand, administration of CT via the intraperitoneal route was notably less effective, in terms of both the rapidity of onset as well as the extent of inhibition observed.

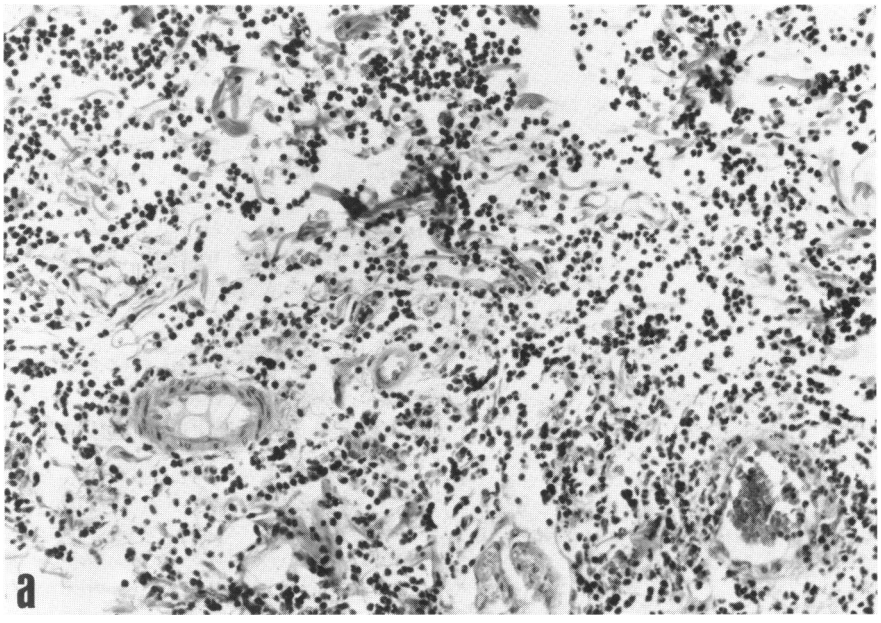
Effects of CT Administration on Con A-Induced Cutaneous Inflammation

The ability of Con A to activate T-lymphocytes (and basophils) has been demonstrated *in vivo* as well as *in vitro*; intradermal injections of Con A induce a diffuse, local inflammatory reaction, predominantly mononuclear in character, and resembling the classic tuberculin-type, delayed hypersensitivity reaction (*7-9*). Therefore, we examined the ability of CT to inhibit Con A-induced cutaneous inflammation, as might be expected if CT inhibited leucocyte activation *in vivo* as well as *in vitro*.

Con A (10 μg) was injected at duplicate sites in rabbits receiving CT according to protocols A (three animals), B (three animals) or C (three animals), and also into non-CT treated controls (three animals). Both schedules of intravenous CT administration (A and B) effectively inhibited the Con A-induced inflammatory response (Fig. 2), whereas intraperitoneal administration gave little, if any, inhibition (Table 1). These results paralleled those obtained in the preceding experiment, again indicating that, whereas intravenous administration of CT effectively blocked *in vivo* the lymphocyte's responsiveness to Con A, intraperitoneal administration was decidedly less effective. Based on these observations, protocol A was employed in subsequent experiments.

Differential Effects of CT Administration on Delayed Hypersensitivity and Immune Complex Reactions in the Skin

The direct active Arthus reaction, induced by injecting antigen intradermally into an actively immunized animal, consists in part of a necrotizing vasculitis mediated by immune complexes of antigen, antibody and complement, localized within the vessel wall, and, in part, of a diffuse cutaneous leucocytic infiltrate (similar to that produced by Con A) considered to represent a delayed-type hypersensitivity response (*10*). Importantly, the vasculitis



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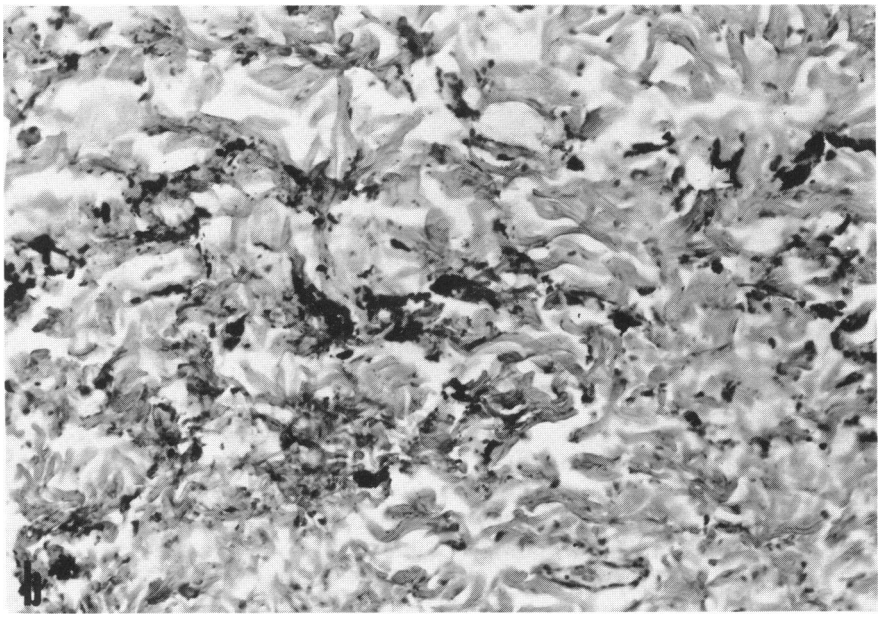


(b)

FIGURE 2. Local inflammatory response to intradermal injections of Con A in the rabbit: (*a*) diffuse inflammatory infiltrate observed in the collagenous dermis of a normal rabbit 24 hr following injection of 10 μg Con A (× 155); (*b*) absence of any inflammatory reaction 24 hr following injection of 10 μg Con A in a CT-treated rabbit (protocol A). The black particulate material present in the center of the field is sterile india ink which had been admixed with the Con A in order to positively identify the injection site (× 155).

Table 1. Effects of CT administration on Con A-induced cutaneous inflammation.

Rabbit no.	CT administration protocols	Skin test grade (duplicate sites)[a]	
293	(A) 40 μg/kg IV, day 0,	+	++
294	20 μg/kg, 48 hr later	+	0
295		+	0
296	(B) 20 μg/kg IV, daily	0	0
297	for 4 days	+	0
298		0	0
299	(C) 40 μg/kg IP, day 0,	+++	++
300	20 μg/kg, 48 hr later	+++	++
288		+++	+++
249	None	++++	++
251		+++	++++
252		++++	++++

[a]Intensity of inflammation graded from 0 (no inflammation) to ++++ (extensive inflammation).

Table 2. Differential effects of CT administration on delayed hypersensitivity and immune complex reactions in the skin.[a]

Rabbit no.	Total CT, μg/kg, body weight	Arthus reaction (50 μg BSA)		Delayed hypersensitivity (10 μg Con A)
		Vasculitis	Diffuse inflammation	
133	0	++++	++++	++++
134	0	++++	++	++++
138	0	+++++	+++	++++
140	0	+++	++	+++
135	60	++++	0	++
136	60	++++	0	++
137	60	++++	0	++
139	60	+++++	0	0
141	60	++++	0	+

[a]Intensity of inflammation graded from 0 (no inflammation) to +++++ (extensive inflammation).

does not require participation by sensitized T-lymphocytes, but instead is mediated by immune complexes, together with homocytotropic antibodies which participate in initiation of complex formation, and neutrophils which respond to the complement-mediated chemotactic stimulus (*10*). Thus, it can be readily produced in animals immunized passively with antibody alone.

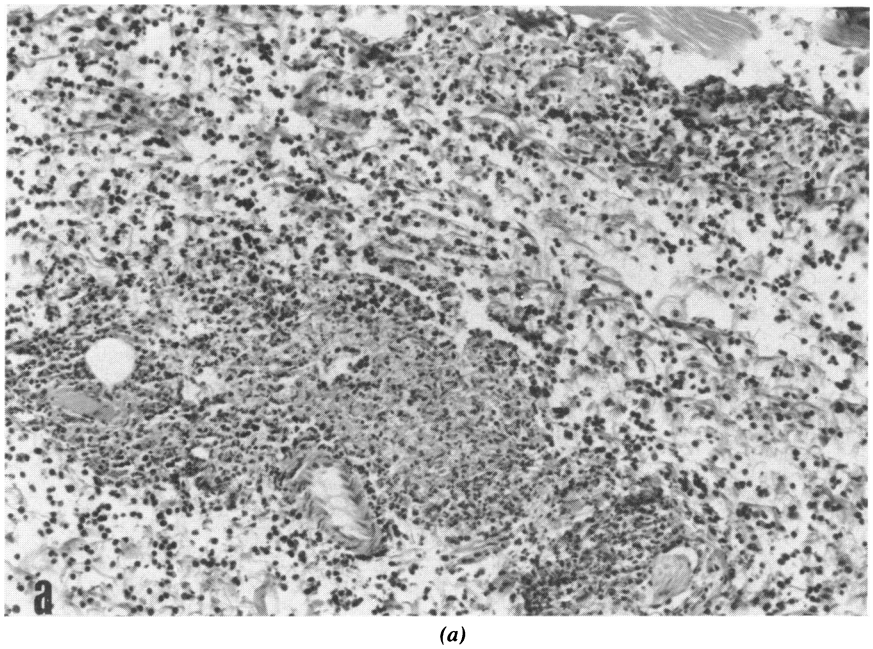
Using both the BSA-induced Arthus reaction (in BSA-immunized rabbits) as well as the Con A skin test, we were thus able to compare the effects of CT administration upon an immune complex-mediated vasculitis with its effects upon delayed-type hypersensitivity reactions.

As in the previous experiment, intravenous administration of CT inhibited the inflammatory response to Con A (Table 2). Of particular interest, however, was its effect upon the active Arthus reaction, where it inhibited the diffuse inflammatory or delayed hypersensitivity component of the Arthus, but not the immune complex-mediated vasculitis (Fig. 3). Thus, these findings argue eloquently for a selective inhibitory effect by CT upon effector lymphocytes of the delayed-type hypersensitivity reaction, while leaving simple immune-complex mediated reactions intact.

Effects of CT upon the Immunologic Pulmonary Injury Associated with Inhalation of Antigen plus a Polyclonal Cell Activator

We have recently reported that polyclonal cell activators such as Con A and phytohemagglutinin induce a diffuse interstitial pneumonitis when inhaled in aerosol form (*2*). More importantly, they also exert a pronounced enhancing effect upon immune complex injury involving inhaled antigen, humoral antibody and complement; such injury is severe and is characterized by foci of frank parenchymal necrosis (*3*). We have proposed that this enhancement may be mediated, at least in part, through polyclonal activation of leucocytes within the lung by Con A, leading in turn to the interstitial inflammation typically observed following inhalation of Con A (*3*). In particular, Con A is known to cause T-lymphocytes to release vascular permeability factors (*11*) *in vitro*, and to cause mast cells and basophils to release vasoactive amines *in vitro* (*12*); inhaled Con A could presumably be having the same effect on these cells *in vivo*. In any event, inhaled Con A might alter the cellular barriers which normally isolate intra-alveolar antigen from humoral antibody and complement. If this hypothesis is true, then administration of CT should block both Con A-induced interstitial pulmonary inflammation and immune-complex mediated parenchymal necrosis, even though, as seen in the preceding experiment, CT has no apparent effect on simple immune complex-mediated reactions such as the Arthus vasculitis.

The BSA-immunized rabbits were divided into two groups of five each; one group was given CT intravenously, according to protocol A; the other group did not receive CT but served as a control. All animals were challenged with Con A/BSA aerosols at 24, 48, and 72 hr following the initial CT injection and were sacrificed at 96 hr. Histologic examinations of lung tissues revealed that both the extent and severity of inflammation were greatly reduced in the CT-treated animals as compared to the controls (Fig. 4). Of particular importance was the striking reduction in the incidence of immune-complex type



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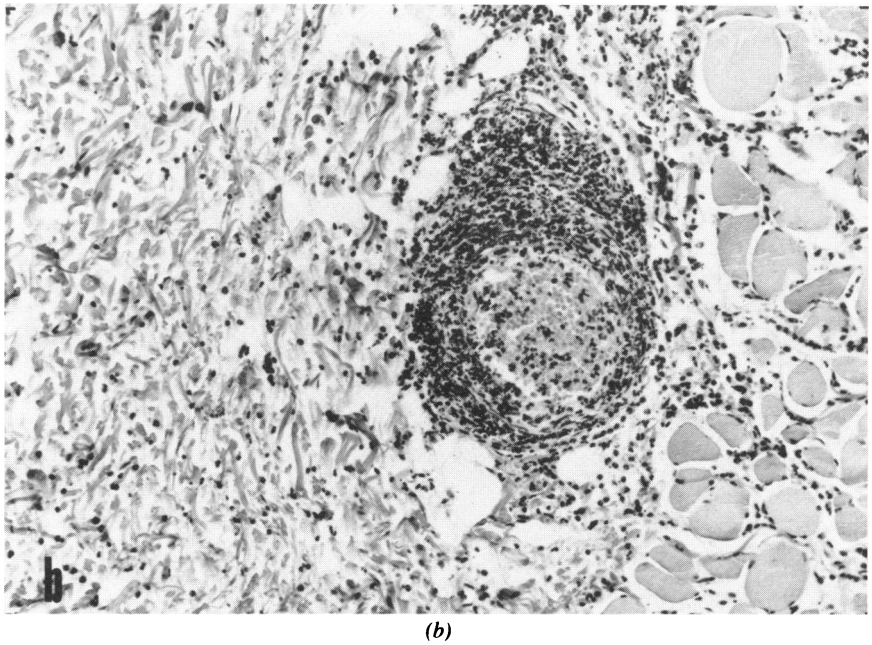
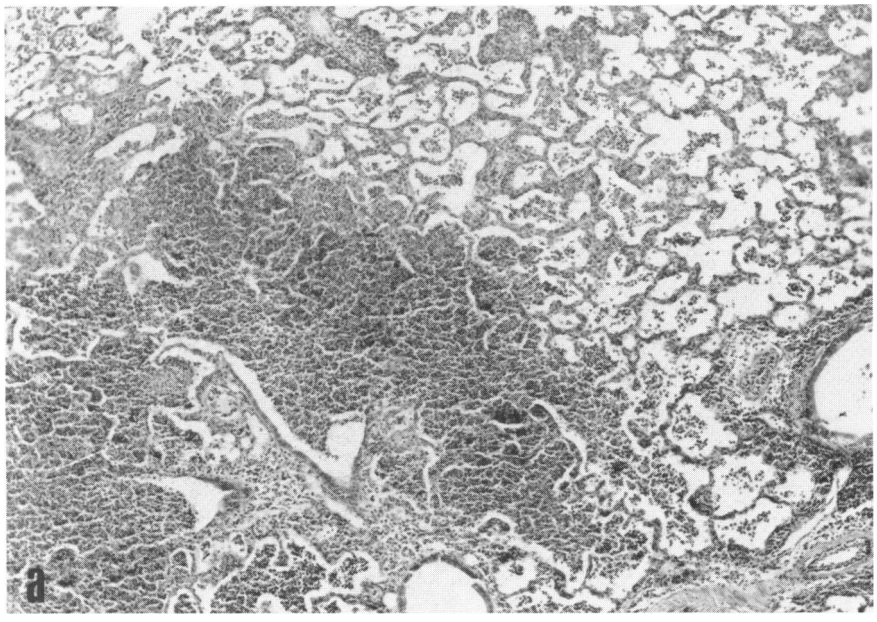


(b)

FIGURE 3. Direct active Arthus reaction to BSA injected intradermally into a BSA-immunized rabbit: (*a*) Arthus reaction in a control animal; notice both the necrotizing vasculitis (mediated by immune complexes containing BSA, anti-BSA antibody and complement) and the diffuse inflammatory infiltrate (representing a delayed hypersensitivity reaction to BSA) (× 155); (*b*) direct active Arthus reaction in a rabbit treated with CT. An intense necrotizing vasculitis is still seen, but the diffuse inflammatory component is notably reduced (× 155).



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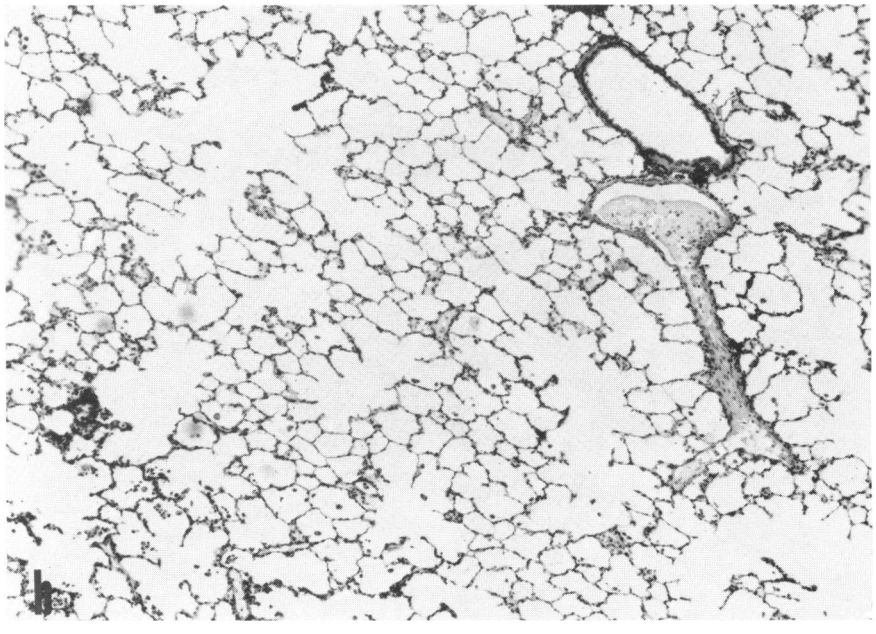

(*b*)

FIGURE 4. Pulmonary injury following administration of BSA/Con A aerosols to BSA-immunized rabbits: (*a*) severe interstitial pneumonitis with areas of frank parenchymal necrosis in a control animal not given CT (× 60); (*b*) inhibition of pulmonary injury in a CT-treated animal. A small focus of interstitial inflammation is seen in the right upper corner (× 60).

Table 3. CT-Induced suppression of immunologic pulmonary injury in BSA-immunized rabbits challenged with BSA/Con A aerosol.[a]

Rabbit no.	CT, μg/kg body weight	Lung injury[b]								Lymphocyte cAMP, pmole/10^6 cells
		MeL[a]	RUL	RML	RLL_h	RLL_p	LUL	LLL_h	LLL_p	
131	0	0	[++++]	[+++]	+	0	[+++]	++	0	
133	0	0	0	[++]	++	+	+	0	0	
134	0	[+++]	+	++++	+	0	[++++]	0	0	7.0 ± 1.2
138	0	[+++]	[+++]	[+++++]	[++]	++	[+++++]	+	0	
140	0	0	+	[+++]	0	0	[++++]	0	+	
135	60	+	0	++	0	0	+	0	0	
136	60	0	0	+	++	0	0	ND	0	
137	60	[++++]	++	++++	++	0	[++++]	+	0	18.6 ± 2.4
139	60	+	0	+	0	+	0	+	0	$p = 0.002$
141	60	0	0	++	0	0	+++	0	0	

[a] Lesions graded from 0 (no injury) to +++++ (severe injury). Lobes containing one or more foci of parenchymal necrosis are enclosed in boxes. ND = not examined.

[b] Sites of injury: MeL = mediastinal lobe; RUL = right upper lobe; RML = right middle lobe; RLL_h = hilar portion of right lower lobe; RLL_p = peripheral portion of right lower lobe; LUL = left upper lobe; LLL_h = hilar portion of left lower lobe; LLL_p = peripheral portion of left lower lobe.

necrosis, with such lesions being observed in only two lobes from a single animal receiving CT, whereas such lesions were present in a total of 14 lobes of the control group, with all animals being affected (Table 3).

In vitro studies have shown CT to act through adenylate cyclase to increase cellular cAMP levels. In order to look for a similar effect *in vivo*, we obtained daily bleedings from the five rabbits receiving CT via schedule A, as well as from the five controls, harvested the lymphocytes and measured their cAMP levels by radioimmunoassay (Table 3). The average cAMP concentration in cells obtained following the initial CT injection was 18.6 ± 2.4 pmole/10^6 cells, significantly greater than that in cells from control animals (7.0 ± 1.2 pmoles/10^6 cells, $p = 0.002$).

In some cases, different experimental observations were carried out simultaneously in the same animals, thereby providing a form of internal control and underscoring the mutual relevance of the different phenomena. For example, the selective action of CT upon cell-mediated versus antibody-mediated reactions in the skin, its effects on Con A/BSA aerosol challenge, and on cAMP levels in peripheral blood lymphocytes, were performed simultaneously in animals 131-141 (see Tables 2 and 3). Thus, observations that CT treatment was able to block the immune complex-mediated parenchymal necrosis, but not the Arthus vasculitis, were made simultaneously in individual CT-treated animals.

Discussion

We have previously proposed that inhaled polyclonal cell activators enhance local immune complex injury, in an indirect fashion, through their ability to stimulate pulmonary lymphocytes. These activated lymphocytes could then "trigger" immune complex formation between intra-alveolar antigen and humoral antibody by releasing lymphokine mediators or, possibly, through direct cell-cell cytotoxic reactions. This hypothesis has been critically tested in this study by examining whether selective inhibition of lymphocyte responses to Con A *in vivo* in fact blocked not only cell-mediated interstitial lung injury, but immune complex-mediated parenchymal necrosis as well.

To achieve such a selective inhibition *in vivo*, we elected to use CT for several reasons. First, the ability of CT to inhibit lymphocyte effector functions *in vitro* through its actions as a cAMP agonist is now well documented (*13, 14*). Moreover, we favored CT over other known cAMP agonist since it binds irreversibly to cell membranes, and its cAMP stimulating effects are notably prolonged relative to those of the beta adrenergic stimulators (*15*). In addition, Warren and colleagues (*16*) had previously reported that CT, when administered intravenously to mice, inhibited cell-mediated inflammatory responses to schistosome egg antigen, in association with a doubling of spleen cell cAMP content.

As a first step, we demonstrated that CT administration could inhibit peripheral blood lymphocytes. Lymphocytes harvested from CT-treated animals were shown to have impaired proliferative responses when treated with optimal concentrations of Con A *in vitro*, and the local *in vivo* inflammatory response to intradermal injections of Con A was also suppressed. Second, we showed that the effect of CT on immune effector responses was selective, by demonstrating that it inhibited both the cell-mediated response to Con A and the classic delayed-type hypersensitivity response to the protein antigen,

BSA, but that it failed to inhibit the cutaneous Arthus vasculitis mediated by antigen, antibody and complement. The latter is particularly noteworthy, since there is good evidence that this vasculitis is triggered by an anaphylactic reaction involving the release of vasoactive amines (and possibly platelet activating factor) from mast cells and basophils sensitized with homocytropic antibody (*3*). Thus, the inability of CT to block the Arthus vasculitis also argues against any significant inhibition of basophil or mast cells by CT in these studies.

The present demonstration that CT administration blocks both the interstitial pneumonitis [associated with injury by Con A alone (*3*)] and the immune complex-associated necrotizing injury supports the hypothesis that cell-mediated lung injury acts to enhance or facilitate immune complex formation in the lung. Stimulation of T-lymphocytes appears to be the critical initiating event for the following reasons: Con A is known to directly stimulate T-cells (but not B-cells) (*17*); T-lymphocytes are normally present in the lung (*18*); Con A has little effect upon macrophages (*19*); and we have now demonstrated that inhibition of immune complex lung disease by CT is associated with inhibition of T-lymphocyte functions, in the absence of demonstrable effects on the immune complex-mediated, basophil/mast cell initiated Arthus vasculitis.

It should be noted that in some instances the cutaneous response to Con A and also the production of interstitial pneumonitis were only partially blocked by CT, yet inhibition of immune-complex injury was striking. Thus, initiation of immune complex injury seems dependent upon the extent of cell-mediated injury produced. Similarly, this finding may also explain the relative inability of BSA alone to initiate immune complex injury (although cell-mediated responses to BSA were observed by the direct active Arthur skin test) since only a small percentage of bronchoalveolar T-lymphocytes would theoretically specifically recognize BSA antigen. In contrast, all or most T-lymphocytes should respond to polyclonal activation by Con A except, of course, in animals treated with CT.

Demonstrations of signicant increases in cAMP in peripheral lymphocytes from CT-treated animals indicate that CT is exerting its inhibitory effects *in vivo* through stimulation of intracellular "second messengers." This finding parallels those changes observed when such cells are treated with CT *in vitro* (*13, 14*), and the 2.7-fold increase observed here agrees well with the value of 2.1 that Warren and colleagues observed in mouse spleen cells following a single injection of CT (*16*).

In conclusion, these studies call attention to the possible importance of polyclonal cell activators which, when present in the environment, may play a critical role in initiating environmental lung disease. Such polyclonal activators include not only a vast array of plant proteins, including lectins such as that employed in this study, but also endotoxins, enzymes, and even simple inorganic chemicals (*20*). In addition, this study additionally emphasizes the importance of regulation, by intracellular mediators such as cAMP, of immune reactive cells, and in particular their ability to respond in the production of immune injury. Consideration of such factors may prove useful in future clinical studies of environmental lung disease, and may facilitate further definition of risk factors both at the individual as well as environmental level.

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